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Genetics

Nitrogen control in *Salmonella*: Regulation by the *glnR* and *glnF* gene products

(regulation of protein synthesis/positive control/glutamine synthetase/amino acid transport components)

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ABSTRACT **The product of the *glnR* gene is required for nitrogen regulation of the synthesis of glutamine synthetase (Gln synthetase) [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] and two periplasmic transport proteins that are subject to nitrogen control in *Salmonella*. Strains with mutations to loss of function of the *glnR* product [e.g., a strain with a Tn10 insertion or one with an ICR-induced (frameshift) mutation in *glnR*] have about 3% as much Gln synthetase as a fully derepressed wild-type strain and are unable to increase synthesis of this enzyme or periplasmic transport proteins in response to nitrogen limitation. The structural gene for Gln synthetase, *glnA*, and those for the periplasmic transport proteins are unlinked on the chromosome; thus, *glnR* appears to encode a diffusible positive regulatory element. Consistent with this, the mutant *glnR* allele is recessive to the wild-type allele with regard to expression of *glnA* (synthesis of Gln synthetase). Although *glnR* is closely linked to *glnA*, strains with mutations to complete loss of function of the *glnR* product can be distinguished from *glnA* strains by their ability to produce detectable Gln synthetase and to grow in the absence of glutamine. To demonstrate unequivocally that *glnR* is distinct from *glnA*, we have purified and characterized Gln synthetase from a strain with a Tn10 insertion in *glnR*. Because the properties of Gln synthetase from the insertion mutant, most importantly the carboxyl-terminal sequence of amino acids, are the same as those of synthetase from wild type, the Tn10 insertion cannot be in *glnA* (if it were, the carboxyl terminus of Gln synthetase would have to be altered); therefore we conclude that the Tn10 insertion is in a regulatory gene, *glnR*, which is distinct from *glnA*. A model for the function of the *glnR* product together with the previously defined *glnF* product in mediating nitrogen control is discussed.**

In enteric bacteria, synthesis of glutamine synthetase (Gln synthetase) [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] is controlled by availability of nitrogen in the growth medium (1). We previously demonstrated that the product of a positive regulatory gene, *glnF*, is essential for synthesis of this enzyme in *Salmonella* (2). We now have evidence that the product of a second positive regulatory gene, *glnR*, is also required. In addition, both the *glnR* and *glnF* products appear to be required for nitrogen regulation of the synthesis of two periplasmic transport proteins (a glutamine-binding protein and the arginine/lysine/ornithine-binding protein) and, thus, they have pleiotropic effects. Genetic studies indicate that the products of the *glnR* and *glnF* genes function together to mediate nitrogen control.

Magasanik, Tyler, and their colleagues have proposed that Gln synthetase mediates nitrogen control in *Klebsiella aerogenes* and *Escherichia coli* (reviewed in refs. 3 and 4). Specifically, they propose that Gln synthetase functions directly as a genetic regulatory element to control transcription of its own structural gene, *glnA* (autogenous regulation), and that it plays a major role in controlling transcription of genes for other proteins subject to nitrogen control. Part of the evidence for their proposal is that mutations that result in fixed, low-level synthesis of proteins under nitrogen control (which they called the GlnR phenotype) were found to lie within *glnA* (5), as were mutations to constitutive high-level synthesis of the same proteins (GlnC phenotype) (6–9). Location of such mutations within *glnA* was based on three-factor transductional crosses and on complementation analysis. Here we present evidence that a separate regulatory gene, *glnR*, lies very close to *glnA* and that mutations to complete loss of function of the *glnR* product result in the GlnR phenotype. We propose that all mutations that result in the GlnR (or GlnC) phenotypes may lie within *glnR* rather than *glnA*.

MATERIALS AND METHODS

Materials. The frameshift mutagen ICR 191E (2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino] acridine dihydrochloride) was kindly donated by H. J. Creech. Affi-Gel Blue was obtained from Bio-Rad. Trypsin (EC 3.4.21.4), bovine type XI, treated with diphenyl carbamoyl chloride, 7500–9000 BAEE units/mg, was purchased from Sigma. Snake venom phosphodiesterase (EC 3.1.4.1, 1.5 units/mg) was obtained from Boehringer Mannheim. Carboxypeptidase A (EC 3.4.12.2), treated with phenylmethylsulfonyl fluoride, 51 units/mg, was obtained from Worthington.

Isolation and Genetic Analysis of Strains. All strains were derived from *Salmonella typhimurium* LT2. Phage was P22HT *int201*. Strains with mutations to loss of function of *glnR* were selected in three ways. The first selection was for decrease in one transport function under nitrogen control followed by screening for pleiotropic loss of other transport functions under nitrogen control. Strain SK294, carrying the *glnR138* mutation, was isolated from an ICR-mutagenized culture of strain SK214 (10) by penicillin enrichment for loss of ability to use D-histidine as histidine source, a selection for decreased histidine transport. The second selection was for suppression of *glnF* mutations (2). Spontaneous derivatives that had regained glutamine independence (e.g., strains SK219 and SK220 carrying the *glnR130* and *glnR129* mutations, respectively) were isolated from *glnF*Δ strains at a frequency of $\approx 10^{-6}$. Because these two selections yielded strains with the same phenotypic properties and with mutational lesions closely linked to *glnA*, the third selection was based simply on proximity of *glnR* to *glnA*. Strain SK293 (*glnR137*::Tn10 *hisF645*) was isolated by transducing strain SK35 [*gln*Δ(*AR*)*60 hisF645*] to glutamine independence with pooled tetracycline-resistant clones containing random insertions of the Tn10 transposon in the *Salmonella* chromosome and screening transductants for tetracycline resistance.

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Abbreviations: Gln synthetase, glutamine synthetase; $NaDodSO_4$, sodium dodecyl sulfate.

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In many cases *glnR* mutations were transferred to a new genetic background before being studied. Strain SK398 (*glnR137*::Tn10 *hisF645*) was obtained by transducing strain TA831 (*hisFΔ645*) to tetracycline resistance with phage grown on strain SK293. Linkage of *glnR* mutations to *glnA* was determined by transducing strain SK429 (*glnA188 hisF645*) to glutamine independence and scoring 50 transductants for ability to grow on arginine as sole nitrogen source. Linkage of *glnR137*::Tn10 to *glnA* was also determined by scoring transductants for tetracycline resistance. Strain SK429 is a spontaneous *glnA* strain that produces inactive Gln synthetase antigen, isolated as described (11). Merodiploid strains were constructed and analyzed as described (2).

Assays. Assays for Gln synthetase and amino acid binding protein activities and for levels of the corresponding antigens were done as described (10). Synthetase activities were corrected for "blanks" in the absence of adenosine diphosphate and arsenate, except as noted. Protein was determined by the method of Lowry *et al.* (12) with bovine serum albumin as standard.

Purification of Gln Synthetase. The enzyme was purified from strain SK398 (≈1500 g wet weight) and the wild-type strain TA831 (185 g). Cells were grown in medium E (13) plus histidine and 3 mM glutamine (nitrogen-excess conditions). Synthetase activity in the mutant crude extract (16% that of wild type) could not be accounted for by reversion because <1% of cells from mutant cultures formed large (wild-type) colonies or had become tetracycline sensitive. None of the cells ($<1/10^9$) was a histidine prototroph, indicating that no contaminating cells were in the culture. Purification steps were those described (14) followed by precipitation with acetic acid at pH 5.05 and affinity chromatography on Affi-Gel Blue (as suggested by J. Davis and E. R. Stadtman).

Characterization of Gln Synthetase. A sequence of six residues at the amino terminus of the enzyme was determined by automated Edman degradation on a Beckman 890C sequencer (kindly performed by Alan J. Smith) (15). To determine the residue at position 4, we regenerated the free amino acid from the phenylthiohydantoin derivative by acid hydrolysis (16) and identified it by amino acid analysis. A sequence of seven residues at the carboxyl terminus of the enzyme was determined by digesting it with carboxypeptidase A and identifying the amino acids released on a Durrum D 500 amino acid analyzer (17). Gln synthetase (0.9 mg) was treated with snake venom phosphodiesterase (0.006 unit) at 37°C to remove adenylyl groups (11, 18). Controls were incubated without phosphodiesterase. Gln synthetase (0.15 mg, before and after phosphodiesterase treatment) was subjected to cleavage with trypsin (0.5 μg) under mild conditions similar to those described (19). Microcomplement fixation experiments to determine the immunological similarity of Gln synthetase from strain SK398 to that from wild type were kindly performed by Linda Baumann as described (20).

RESULTS

Phenotype of *glnR* Strains. Properties of strains with mutations to loss of function of the *glnR* product are summarized in Table 1. The *glnR* strains retained the ability to grow in the absence of exogenous glutamine although their growth was stimulated by glutamine. In this way they could be distinguished from both *glnA* and *glnF* strains (Table 1). The *glnR* strains failed to grow on arginine as nitrogen source (data not shown). The *glnR* mutations suppressed mutations to loss of function of the *glnF* product—that is, *glnR glnF* strains grew in the absence of exogenous glutamine whereas *glnF* strains were glutamine auxotrophs. In fact, *glnR glnF* strains had the same properties as *glnR* single-mutant strains.

Gln Synthetase and Binding Protein Activities of *glnR* and *glnF* Strains. Both *glnR* and, as reported (2), *glnF* strains synthesized only small amounts of Gln synthetase and were unable to increase synthesis of this enzyme in response to nitrogen limitation (Table 1). The *glnR* strains had detectable synthetase activity (≈3% as much as a fully derepressed wild-type strain), which apparently is sufficient to allow their growth in the absence of exogenous glutamine. The *glnF* strains had <1% as much synthetase activity as a fully derepressed wild-type strain (ref. 2; Table 1). Both *glnR* and *glnF* strains had low activities of the periplasmic glutamine-binding protein and the periplasmic lysine/arginine/ornithine-binding protein (10) and, unlike wild type, did not increase the levels of these proteins when grown under nitrogen-limiting conditions. For both Gln synthetase and the binding proteins, low levels of activity in *glnR* and *glnF* strains were correlated with low levels of antigen (data not shown). Low levels of arginine transport components in *glnR* strains may contribute to their inability to grow on arginine as nitrogen source (10). Double-mutant strains *glnR glnF* had the same properties as *glnR* single-mutant strains (Table 1).

Table 1. Doubling times and Gln synthetase and binding protein activities in regulatory mutant strains

Strain*	Doubling time, min†			Gln synthetase,‡ μmol/min·mg		Gln-binding protein§		LAO-binding protein¶	
	−Gln	+Gln	+Gln, Arg, Ura, Hyp	N excess	N limiting	N excess	N limiting	N excess	N limiting
TA831 (wild type)	44	43	38	0.15	1.25	4.2	14.3	2.4	28.6
SK398 (*glnR137*::Tn10)	60	48	40	0.03	0.03	1.6	1.0	0	1.0
SK294 (*glnR138*)	55	45	40	0.03	0.04	1.2	0.9	1.4	0
SK99 (*glnF75*)	≥320	57	43	<0.01	<0.01	1.4	1.1	0.5	0.8
SK100 (*glnF76*)	≥320	58	43	<0.01	<0.01	1.3	0.9	1.6	0
SK464 (*glnR137*::Tn10 *glnF75*)	57	47	41	0.03	0.03	1.2	0.9	0.3	0.4
SK465 (*glnR138 glnF75*)	59	47	40	0.03	0.04	1.6	0.9	0.5	0

* All strains contain the *hisFΔ645* mutation. Strains SK294 and SK465 also carry the *gln-128* mutation (10).

† Minimal medium (21) without glutamine, with glutamine, or with glutamine and the additional supplements described in footnote‡. Media contained glucose (0.4%) as carbon source and 20 mM NH_4^+.

‡ N excess: minimal glucose medium with 20 mM NH_4^+ and 3 mM glutamine as nitrogen sources. N limiting: 3 mM glutamine as sole nitrogen source. Both media were supplemented with 0.2 mM arginine, uracil, and hypoxanthine (and 2 μM thiamine), major end products that contain a nitrogen atom derived from glutamine, to stimulate the growth rate of mutant strains.

§ pmol/mg dry weight of cells.

¶ Lysine/arginine/ornithine-binding protein; pmol/mg dry weight cells, calculated as described (10). Total arginine binding by the LAO and other arginine-binding proteins under N-excess and N-limiting conditions was: TA831, 31.5 and 41.6; SK398, 31.5 and 10.8; SK294, 31.1 and 10.4; SK99, 26.1 and 15.4; SK100, 13.8 and 5.1; SK465, 30.1 and 16.9.

Table 2. Complementation of *glnR* mutations

Strain*	Gln synthetase,† μmol/min·mg
SK290 (*glnR129*/F′*glnR*$^+$)	0.35
SK291 (*glnR130*/F′*glnR*$^+$)	0.34
SK292 (*glnR*$^+$/F′*glnR*$^+$)	0.37
SK215 (*glnR129*)	0.04
SK217 (*glnR130*)	0.04
TA831 (wild type)	0.22
SK178 (*glnΔAR60*/F′*glnR*$^+$)	0.10
SK35 (*glnΔAR60*)	<0.01

* All strains contain the *hisF645* mutation. Strains carrying F′*glnR*$^+$ (F′133 coli) also contain the *argH1823*::Tn10 mutation, which is covered by this episome.

† Not corrected for blank activity. Strains were grown on medium E (13) plus 3 mM glutamine (nitrogen-excess conditions).

Mapping and Complementation of *glnR*. Four *glnR* mutations that were studied extensively were 66–83% linked by P22-mediated transduction to the *glnA188* mutation; the linkage of *glnR137*::Tn10 was 71%. Complementation of *glnR*$^-$ mutations with the *E. coli* episome F′133 suggested that the wild-type allele was dominant and, therefore, that *glnR* encodes a diffusible product, consistent with its having a pleiotropic role in nitrogen regulation. A *glnR*$^-$/*glnR*$^+$ merodiploid (lines 1 and 2, Table 2) has as much Gln synthetase activity as a *glnR*$^+$/*glnR*$^+$ strain (line 3) and considerably more than the sum of the activities (0.14) expected if the chromosome (lines 4 and 5) and the episome (line 7) were functioning independently. (Independent function would have been expected if *glnR* were a *cis*-acting regulatory site that controlled expression of the adjacent *glnA* gene.)

Properties of Gln Synthetase from SK398 (*glnR137*::Tn10). The growth behavior and Gln synthetase specific activities of *glnR* strains (Table 1) distinguish them from *glnA* strains. To demonstrate unequivocally that *glnR* is distinct from *glnA*, we purified Gln synthetase (Table 3) from strain SK398 (*glnR137*::Tn10) and compared its properties with those of the synthetase from strain TA831 (wild type). Recoveries of activity suggested that Gln synthetase from the mutant was stable to heat, acetone, and acid pH, as was the enzyme from wild type. Consistent with the relative specific activities in extracts of mutant (0.03) compared to those of wild type (0.19), 1600-fold purification of the enzyme was required from the mutant strain whereas only 300-fold was required from wild type. After treatment of the purified enzymes with snake venom phosphodiesterase to remove adenylyl groups, the same specific activity was achieved. Mobilities on sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis (Fig. 1) indicated that the subunit molecular weight of Gln synthetase from the mutant was the same as that from wild type, as were the molecular weights of fragments produced by mild proteolytic cleavage of the synthetase with trypsin (19). Gln synthetase from mutant and wild type could not be distinguished immunologically by microcomplement fixation, which is very sensitive to small sequence differences between homologous proteins (22). In summary, Gln synthetase from mutant and wild type could not be distinguished on the basis of specific activity, stability during purification, subunit molecular weight, cleavage by trypsin, or immunological behavior, indicating that the Tn10 element in the mutant was not inserted in the middle of the *glnA* gene. [Insertion of the Tn10 element into a structural gene completely disrupts transcription and translation of the gene and thus causes complete loss of function of the gene product (23).]

Table 3. Purification of Gln synthetase from strains SK398 (*glnR137*::Tn10) and TA831 (wild type)

Fraction	Specific activity, μmol/min·mg		Recovery, %	
	TA831	SK398	TA831	SK398
Crude*	0.19	0.03	100	100
$(NH_4)_2SO_4$ ppt.	0.67	0.09	97	60
Heated supernatant	2.3	0.18	119	63
Acetone ppt.	6.0	1.3	98	56
Acid ppt.	13.9	3.1	76	46
Affi-Gel Blue eluate†	57.2	47.8	53	36
Phosphodiesterase-treated‡	48.0	50.1		

* Total units were 3645 for TA831 and 2937 for SK398.

† Samples were applied in the presence of 10 mM glutamate, and Gln synthetase was eluted in the presence of 10 mM glutamate, 0.1 M KCl, and 10 mM ADP.

‡ As described in *Materials and Methods*. The reaction was complete in 2 hr. The ratio of activities $+Mg^{2+}/-Mg^{2+}$ (18) for Gln synthetase from TA831 increased from 0.5 to 1.4 after phosphodiesterase treatment, indicating that the synthetase was partially adenylylated (21), whereas the ratio for strain SK398 was 1.4 and did not change. Adenylylated Gln synthetase is the less active form (18).

The sequence of amino acids at the amino terminus of Gln synthetase from the mutant was the same as that of wild type (NH_2Ser-Ala-Glu-His-Val-Leu-). [With the exception of histidine at position 4, the sequence is the same as that reported for Gln synthetase from *E. coli* (17).] The carboxyl-terminal set of seven amino acids released by digestion with 10 μg of carboxypeptidase A and the pattern of release of the first five

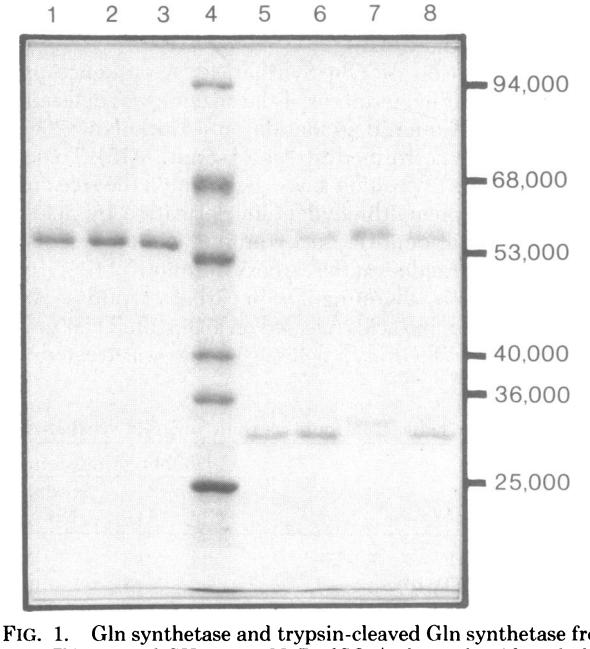

FIG. 1. Gln synthetase and trypsin-cleaved Gln synthetase from strains TA831 and SK398 on $NaDodSO_4$/polyacrylamide gel electrophoresis (9%). Molecular weights of standards (well 4) (10) are indicated at the right. All other wells (numbered at the top) contain ≈ 0.3 μg of protein. Wells 1 and 2 contain phosphodiesterase-treated Gln synthetase from strains TA831 and SK398, respectively; well 3 contains a mixture of phosphodiesterase-treated synthetase from the two sources; wells 5–8 contain trypsin-cleaved synthetase from TA831 (phosphodiesterase-treated), SK398 (phosphodiesterase-treated), TA831, and SK398, respectively. The upper band is uncleaved Gln synthetase. The lower band (apparent molecular weight ≈31,000) is the larger trypsin fragment. The smaller fragment ran to the dye front. The upper bands of the doublets observed for Gln synthetase and the larger trypsin fragment of the synthetase from TA831 (well 7) are adenylylated subunit and fragment, respectively (19).

Table 4. Digestion of Gln synthetase with carboxypeptidase A*

Amino acid	Carboxypeptidase A, μg						
	0.01	0.02	0.04	0.08	0.16	1.0	10.0†
TA831							
Val	0.60	0.77	0.75	0.80	0.77	0.81	0.91
Ser	—	0.06	0.15	0.29	0.54	0.79	0.95
Tyr	—	0.06	0.23	0.58	1.0	1.6	1.8
Leu	—	—	—	0.07	0.22	0.76	0.93
Glu	—	—	—	—	—	—	0.15
Phe	—	—	—	—	—	—	0.11
SK398							
Val	0.52	0.72	0.80	0.80	0.80	0.81	0.87
Ser	—	0.07	0.14	0.27	0.49	0.78	0.92
Tyr	—	0.07	0.25	0.55	0.97	1.6	1.71
Leu	—	—	—	0.07	0.17	0.77	0.88
Glu	—	—	—	—	—	—	0.18
Phe	—	—	—	—	—	—	0.12

* Digestion at 37°C for 60 min. Each digestion mixture (0.07 ml final volume, pH 7.4) contained 0.25 mg of Gln synthetase, 21 mM $NaHCO_3$, 7 mM EDTA, 0.085% $NaDodSO_4$, and 0.1% LiCl. Gln synthetase was preincubated with $NaDodSO_4$ and EDTA for 15 min at 45°C before addition of carboxypeptidase A. No free amino acids were found in untreated Gln synthetase controls or in carboxypeptidase A controls. Values are in nmol of amino acid released per nmol of Gln synthetase subunit, based on a molecular weight of 50,000 (17). The sequence inferred, -Phe-Glu-Leu-Tyr-Tyr-Ser-ValCOOH, is based, in part, on the fact that carboxypeptidase A releases Tyr rapidly, Ser slowly, Glu very slowly, and Phe rapidly (24).

† Gln synthetase: 0.5 mg from TA831 and 0.65 mg from SK398 in a digestion mixture of 0.14 ml.

residues with limiting amounts of carboxypeptidase A were the same for Gln synthetase from mutant and wild type (Table 4). The sequence inferred was Phe-Glu-Leu-Tyr-Tyr-Ser-Val-COO^-. [The sequence is the same as one of those proposed for Gln synthetase from *E. coli* (17).] If the Tn10 element in the mutant had been inserted within *glnA*, even at the end of the gene, the carboxyl-terminal sequence of the synthetase from the mutant should have been altered because transcription of the beginning of the *glnA* gene would have been disrupted at some point by transcription into the Tn10 element. The carboxyl terminus of Gln synthetase from the *glnR*::Tn10 strain was *not* altered and, therefore, the transposon must be inserted in a gene distinct from *glnA*, which we have called *glnR*. [The probability that a sequence of seven amino acids at the carboxyl terminus of Gln synthetase from the mutant would have been the same as that from wild type at random is $(1/20)^7$ or 10^{-9}.]

DISCUSSION

We have identified a gene, *glnR*, with an essential role in nitrogen regulation in *Salmonella* and have presented evidence that it is distinct from *glnA* (although closely linked to *glnA*). Merodiploid analysis with an *E. coli* episome suggests that a *glnR* gene is present in *E. coli* at a chromosomal position homologous to that in *Salmonella*.

The *glnR* gene product appears to be a positive regulatory element with pleiotropic function. Mutations that cause loss of function of this product result in synthesis of low, unregulated levels of Gln synthetase and of amino acid transport proteins that are subject to nitrogen control in *Salmonella*. Residual levels of Gln synthetase in *glnR* strains are sufficient to allow their growth in the absence of exogenous glutamine. Mutations to loss of function of a second positive regulatory element, the *glnF* product, have similar consequences except that Gln synthetase levels in *glnF* strains are even lower than those in *glnR* strains and, therefore, *glnF* strains are glutamine auxotrophs.†

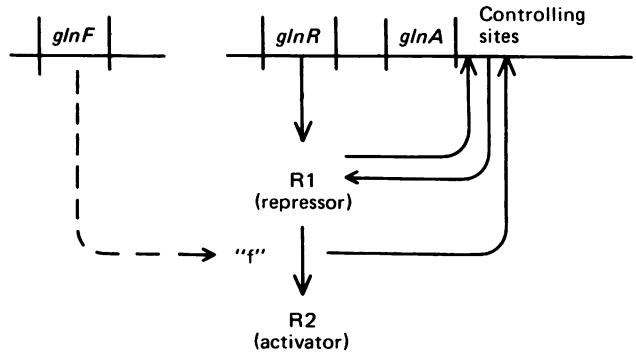

FIG. 2. Working model for control of *glnA* transcription by the *glnR* and *glnF* products. The product of the *glnR* gene exists in two conformations: R1, which represses transcription of *glnA*, and R2, which activates transcription of *glnA* (and other genes under nitrogen control). The *glnF* product, which may catalyze formation of a low molecular weight signal of nitrogen deficiency "f," leads to formation of R2, the activator form. The order of genes in the *glnA* region is arbitrary.

Genetic studies suggest that the *glnR* and *glnF* products function together to mediate nitrogen control and that the *glnR* product has negative regulatory character as well as positive character. One indication that the *glnR* and *glnF* products function together was the initially puzzling observation that mutations to complete loss of function of *glnF* are suppressed by mutations to loss of function of *glnR*. That is, if one selects glutamine-independent derivatives of *glnF* strains, many of them have acquired second mutations to loss of function of *glnR*. This result is consistent with a model for nitrogen control in which the product of the *glnF* gene (directly or indirectly) modifies the product of the *glnR* gene to convert it to a form with positive regulatory character (Fig. 2). In the absence of any *glnF* product, the *glnR* product apparently has exclusively negative regulatory character (at least with regard to *glnA* expression); consequently, a *glnF* strain that lacks the *glnR* product (*glnF glnR*) synthesizes more Gln synthetase and grows better than its parent strain (*glnF*). The *glnF glnR* strains have properties identical with those of *glnR* single-mutant strains. (Residual levels of *glnA* transcription in *glnR* strains may be controlled by another regulatory mechanism or may be due to unregulated transcription by RNA polymerase alone.) The dual positive and negative character proposed for the *glnR* product (Fig. 2) has a precedent in similar dual character of the *araC* product (25). Gaillardin and Magasanik (5) previously proposed a model for nitrogen regulation involving positive and negative control by Gln synthetase as the genetic regulatory element.

Apart from the partial negative character of the *glnR* product, which can be detected in a *glnF* background, the *glnR-glnF* system is a positive regulatory system. As discussed above, mutations to loss of function of either gene product cause a pleiotropic decrease in ability to synthesize proteins under nitrogen control. Thus, there is a striking analogy between the *glnR-glnF* system for nitrogen control and the *crp-cya* system for carbon and energy regulation (26). If this analogy extends to mechanism, *glnR* would encode a macromolecular regulator of transcription and *glnF* would encode an enzyme that cata-

† The following alternative interpretations of our data have not been excluded. (*i*) Mutations in *glnR* may reduce *glnA* expression by polarity. (*ii*) Gln synthetase, the *glnA* gene product, may regulate expression of other genes under nitrogen control although its own synthesis is controlled by *glnF* and *glnR*, as we have proposed.

lyzes synthesis of a low molecular weight coregulator of transcription—a signal of nitrogen deficiency (Fig. 2). When bound to the low molecular weight signal, the *glnR* product would function as an activator of transcription for genes subject to nitrogen control; in the absence of the nitrogen-deficiency signal the *glnR* product would function as an inhibitor (repressor) of transcription, at least for *glnA*. (Note that a model in which *glnR* encodes the macromolecular regulator of transcription accounts for the fact that the properties of *glnR glnF* strains are the same as those of *glnR* strains.)

Magasanik, Tyler, and their colleagues have proposed that nitrogen regulation is mediated directly by Gln synthetase (reviewed in refs. 3 and 4) and that the function of the enzyme as a regulator of transcription depends on its state of covalent modification (3). Several lines of evidence were presented in support of this model. First, mutations that were reported to alter the degree of covalent modification of Gln synthetase had a major effect on synthesis of Gln synthetase and other proteins under nitrogen control in *Klebsiella* (3). We were unable to confirm this finding in *Salmonella* (21). Second, mutations that caused the GlnR and the GlnC phenotypes in *Klebsiella* (see introduction) were reported to lie within *glnA*, the structural gene for Gln synthetase. Our data indicate that there is a separate gene, *glnR*, very near *glnA* on the *Salmonella* chromosome and that mutations to loss of function of *glnR* result in the GlnR phenotype. Because the *glnF* gene has been identified in *Klebsiella* (5), it is likely that there is also a *glnR* gene in this organism. We think that mutations causing the GlnR phenotype, which were isolated as suppressors of *glnF* (5), may lie within *glnR* and not *glnA*.

Note Added in Proof. Effects of the *glnR* product on expression of transport genes appear to be direct. Newly isolated mutations near *glnA* (presumably in a *cis*-acting site *glnI*) result in high-level constitutive synthesis of Gln synthetase in a *glnR*$^-$ background; these mutations do not, however, restore high-level synthesis of the glutamine- or arginine/lysine/ornithine-binding proteins. Thus, regulatory effects of the *glnR* product on synthesis of binding proteins do not appear to be mediated through Gln synthetase.

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